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**TITLE: DEVELOPMENT OF SAFE, EFFECTIVE VACCINES FOR DENGUE VIRUS  
DISEASE BY RECOMBINANT BACULOVIRUS**

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**CONTRACTING ORGANIZATION: National Institutes of Health (NIH)  
Department of Health & Human Services  
Bethesda, Maryland 20205**

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## FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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In our previous Annual Report, covering the period 3/1/91-2/28/92, we described our finding that cells infected with recombinant baculovirus expressing the 80% N-terminal portion of the dengue virus envelope (E) glycoprotein secreted the 80%E protein into the surrounding medium. This property was unique for recombinant baculoviruses containing the dengue E gene constructed in this laboratory. Viruses containing this E fragment from dengue type 2, 3 or 4 were designated b(DEN2, 80%E), b(DEN3, 80%E) or b(DEN4, 80%E), respectively. Mice inoculated with the secreted DEN4 80%E protein in partially concentrated medium developed a stronger antibody response to DEN4 E, as determined by Western blotting, than did mice inoculated with lysates of cells infected with b(DEN4, 80%E) or with previously constructed recombinants b(DEN4, 93%E), b(DEN4, C-preM-E-NS1-NS2A), or b(DEN4, RSVG-E). Since the dengue virus protein products of the latter two recombinants appeared to partially protect rhesus monkeys against challenge with wild-type DEN4 virus in an earlier experiment, we decided to test b(DEN4, 80%E) in a second monkey experiment, to see if we could obtain a more convincing protective response.

A total of 16 rhesus monkeys were inoculated intramuscularly with baculovirus products. Two control monkeys received a lysate of cells infected with wild-type baculovirus, and two received medium from the same cells. Four monkeys were inoculated with a lysate of cells infected with b(DEN4, 80%E), four with medium from these cells, and four with a lysate of cells infected with b(DEN4, 93%E), which does not secrete its E product. Each monkey was inoculated three times with 2 ml of baculovirus product; boosting was done at 4 weeks and 13 weeks. All monkeys were challenged at 16 weeks by subcutaneous injection of  $10^5$  pfu of DEN4 strain 341750, provided as a lyophilized preparation from WRAIR (1/86). Monkeys were bled during the course of immunization to evaluate their immune response and after challenge to assess viremia.

The antibody response to immunization, determined by 50% plaque reduction neutralization using pre-challenge sera, was very poor. One of four monkey recipients of b(DEN4, 93%E) lysate had a titer of 1:10, two animals which received b(DEN4, 80%E) lysate had titers of 1:10, and two monkeys immunized with b(DEN4, 80%E) medium had titers of 1:10 and two had titers of 1:20. All other monkeys, including controls, were negative.

Tests for post-challenge viremia, using amplification on C6/36 mosquito cells followed by C6/36 plaquing, failed to demonstrate viremia. Control animals immunized with wild-type baculovirus material were positive on only a few scattered days, and when the amplification and plaquing were repeated for this group, plaques were again obtained on scattered days, but these differed from the first result. The cause of this failure is not known. We can draw no conclusion from this experiment as to the protective efficacy of these baculovirus products in monkeys, but it is evident that they are only weakly immunogenic.

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